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Research Article

Pathology and Electron Microscopy of *Mycoplasma synoviae* Infection

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Abstract

In this study the investigation on cause of lameness due to *Mycoplasma synoviae* (MS) infection in poultry farms in Telangana and Maharashtra states were carried out. Detailed flock history of each affected flock was collected, and necropsies of two to three ailing sacrificed birds and dead birds were conducted. Diagnosis of MS was done by serology, PCR, electron microscopy and histopathology. In this study, nineteen parent flocks, two commercial layer (CL) flocks showing signs of lameness were considered. Lameness due to hock joint and foot pad swelling was a characteristic clinical sign particularly observed in BP (broiler parent) male birds. Female birds, although serologically positive for MS, did not show signs of lameness. The morbidity from 0.3 to 100 percent in male BP flocks and mortality up to 10 percent was reported. In MS-affected female CL birds, lameness along with hock joint swelling as well as paleness of the comb and breast blister was a consistently feature. Grossly, severe unilateral or bilateral swelling of hock joint/ footpad joint with turbid to caseous exudate in most birds and blood-tinged fluid in a few birds and breast blister in male BP birds with less severe lesions in female BP birds. In CL birds, the gross lesions were similar to BP birds but showed less severity, and anaemia was constantly observed. Histopathologically, in addition to MS-specific lesions, perivascular lymphoid infiltration around blood vessels in the tendon sheath, liver, and brain as well as multinucleated giant cells in the tendon was observed. Mild tracheitis and interstitial and alveolar pneumonia along with heterophilic and lymphocytic infiltration in the heart was observed. In the ultra-structural study, six out of seven tendon specimens showed mycoplasma-like organisms (MLO) in the tendons and joint fluid. Among twenty one flocks affected with tenosynovitis, samples from three flocks (one CL and two BP) were detected by conventional PCR.

Keywords: Broiler Parent; Diagnosis; Electron microscopy; Gross Pathology; Histopathology; *Mycoplasma synoviae*; PCR

Introduction

Tenosynovitis is a major cause of leg weakness in meat-type chickens. In India, although, avian reo virus (ARV) is considered to be the principal cause of this condition in literature [1], however, due to the widespread use of the ARV vaccine, the ARV-associated tenosynovitis has declined considerably as indicated in field surveys and the response of tenosynovitis to the anti mycoplasma drug in the field, and appears to be replaced by *Mycoplasma synoviae* (MS) as a cause of lameness due to tenosynovitis, particularly in broiler parent (BP) and commercial layer (CL) flocks. Mycoplasmas are associated with chronic respiratory disease due to *Mycoplasma gallisepticum* and infectious synovitis due *to Mycoplasma synoviae* (MS) in chicken. Infectious synovitis due to MS was first described in turkey in 1926 and in chicken in 1936 [2,3].

In view of the increased incidences of hock joint and foot pad swelling along with lameness, particularly in male breeder birds with increased culling rate, the flocks were subjected for detailed study to know the cause of lameness. The clinical data collected from different farms showed lameness in male broiler breeder birds without any apparent clinical sign in female breeder birds. However, in CL flocks, the lameness along with hock joint swelling was observed in female birds also out of the flocks investigated. The serological and molecular studies carried out on the samples collected from these farms revealed infectious synovitis as the important cause of lameness in broiler parents as well as CL flocks.

Necropsy examination of dead birds that died due to infectious synovitis showed breast blister, mild air-sacculitis, peritonitis, swelling of hock and foot pad joints with grey-white to yellowish contents in the abdomen.

In view of the seriousness of the disease, the study was planned to investigate lameness in breeder and CL flocks due to tenosynovitis and know the pathology and electron microscopy study of tendons of the bird showing tenosynovitis.

Materials and Methods

Case history

Investigation of lameness due to tenosynovitis in organized poultry (BP and CL) farms in and around Hyderabad, and other districts in Telangana and Maharashtra states were carried out to know the cause of lameness with regard to MS infection. A few healthy flocks were also included in the study to know the MS infection status in healthy flocks. Detailed information viz. age of affected birds, flock size, morbidity, mortality percentage, and clinical signs were collected and recorded (Tables 1 and 2). None of the flocks investigated for MS were vaccinated against MS.

S. N.	Sample code	Area	Flock size		Age (Wk)	Morbidity (Lameness) (%)	Mortality (%)	Major problem
			Female	Male				
1	AR-1A	Maharashtra	10000	1500	14	0.5	NS	Tenosynovitis (TSS)
2	AR-1B		8000	1200	14	0.4	NS	TSS
3	AR-3	TS	20000	3000	19	0.3	NS	TSS
4	AR-4	TS	30000	4500	21	8	3	TSS and lameness in male birds only
5	AR-5	TS	10,000	1300	21	7	3	TSS
6	AR-6	Maharashtra	5200	675	28	37	5	TSS and lameness in male birds only
7	AR-7	TS	22500	2500	31	10	3	TSS and lameness in male birds only

8	AR-8A	Maharashtra	30000	3900	9.3	30	10	TSS and lameness in male birds only
9	AR-8B		30000	3900	15.3	7	2	TSS and lameness in male birds only
10	AR-8C		30000	3900	21.2	6	1.5	TSS and lameness in male birds only
11	AR-8D		30000	3900	24.3	5	1	TSS and lameness in male birds only
12	AR-8E		30000	3900	47	8	3	TSS and lameness in male birds only
13	AR-9A	Maharashtra	5000	650	17	100	3	TSS and lameness in 100 per cent male birds
14	AR-9B		5000	650	21.4	35	2.5	TSS and lameness in male birds only (TSS occurred at 7 wk. of age and continued up to 23 wk.

Table 1: Details of farms affected with tenosynovitis.

TS-Telangana state.

SN	S. code	Area/District	Type of birds	Flock size		Age (Wk)	Morbidity (Lameness) (%)	Mortality (%)	Major problem
				Female	Male				
15	AR-11	TS	BP	13500	1993	58	1	0.5	TSS
16	AR-12	Maharashtra	BP	10000	1500	12	5	0.5	TSS
17	AR-13	Maharashtra	BP	18000	2340	2	10	3	TSS
18	AR-14	Maharashtra	BP	10000	1300	3.1	7	2	TSS
19	AR-15	Maharashtra	BP	6000	780	8.4	0.5	NS	TSS
1	AR-2	TS	CL	2400	--	15	0.42	NS	TSS
2	AR-10	TS	CL	30000	--	58	5	3	TSS

Table 2: Details of the tenosynovitis affected farms/flocks

BP-Broiler parent; CL-Commercial layer; NS-Non significant; TSS-Tenosynovitis; TS: Telangana state.

Necropsy examination/gross pathology

Necropsy examination was done on two to three sacrificed birds including dead birds showing hock joint swelling, thoroughly examined and gross morphological changes in various visceral organs and joints were noted.

Sample collection

Specimen of tendons (joint), heart, liver, trachea, lung, and brain from tenosynovitis (TSS) affected flocks were collected and preserved in 10 percent neutral buffered formalin (NBF) for fixation. Following fixation, the selected samples were placed in labeled cassettes for processing, paraffin embedding, and cutting at 5 µm thick sections. The tissue sections were stained with hematoxylin and eosin (H&E) following the method described by Kim and co-author [4], then examined under a light microscope fitted with a digital camera, and images were captured.

Serology

The blood samples were collected in serum vacutainer from fifteen broiler breeder farms and two CL farms showing signs of

lameness due to tenosynovitis in order to diagnose MS by serology. After collection of blood in serum vacutainer, the tubes were allowed to stand in slanting position for half an hour and serum was separated and transferred in Eppendorf tube, labeled properly and stored at -20^{0}C until use. Detection of antibody for MS was carried out by ELISA using antibody testing kit (Idexx Laboratories. USA) and performed as per standard protocol given in the Idexx manual. Serum sample value with S/P ratio greater than 0.50 or mean titers with the cutoff point of 1076 and above were considered positive as specified in the Idexx reference guide for MS.

Transmission electron microscopy (TEM)

Samples measuring 1 mm^2 were collected from the affected tendons and fixed in 2.5% glutaraldehyde for transmission electron microscopy (TEM) analysis. Additionally, joint fluid collected from swollen joints was fixed with 2.5 % glutaraldehyde for TEM. After 48 hours of glutaraldehyde fixation, the tissue samples as well as joint fluid were processed for Electron Microscopy (EM) evaluations [5]. All electro-micrographs were analyzed on a TEM analyzer (Hitachi H-7500, Ruska Laboratory, College of Veterinary and animal Sciences, PVNRTVU, Rajendranagar, Hyderabad).

Detection of *Mycoplasma synoviae* by polymerase chain reaction

Sample processing and DNA extraction

Tendon, joint fluid, and lung samples were collected during the necropsy. Pooled tissue inocula were prepared as 10 % weight/ volume suspensions in phosphate buffer saline (pH 7.2) by trituration and three freeze–thaw cycles followed by centrifugation at 1600 x g for 30 min at 4°C and filtration through 0.20 µm filters. Supernatants were stored at –80ºC until further use. DNA was extracted from the filtrates using a DNeasy blood and tissue kit (Qiagen, Valencia, CA) as per the manufacturer's instructions. Extracted nucleic acids were stored at - 20° C until further use. The purity and concentration of the extracted DNA were estimated by UV spectrophotometry (Nanodrop, M/s Thermo Scientific).

Polymerase chain reaction

The polymerase chain reaction was carried out using forward primer MS Link (5-TAC TAT TAG CAG CTA GTG C-3)and reverse primer MS Cons-R (5-AGT AAC CGA TCC GCT TAA T-3) to amplify 350-400 bp of the single copy conserved 5' ends of the vlhA genes [6]. PCR was performed using Platinum Taq DNA Polymerase High Fidelity kit (Invitrogen) as per manufacturer's instructions. The PCR reactions were conducted using the ProFlex™ 3 x 32-well PCR System (Thermo Fisher). The PCR was run with an initial denaturation stage at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 30 sec, primer annealing at 54°C for 30 Sec, and extension at 68°C for 40 sec, and final extension at 68°C for 10 min. The products obtained by PCR for MS were subjected to agarose gel (1%) electrophoresis along with positive control and 100 bp DNA molecular weight marker for each sample. The specificity of PCR products was confirmed by the appearance of the desired band of specific molecular weight (350-400 bp) under a UV transilluminator.

Results

Clinical signs, morbidity and mortality

The birds from a total of nineteen parent (Eighteen BP and one coloured parent) flocks showed signs of lameness (Figure 1) due to the severe unilateral or bilateral swelling of the hock joint and foot pad swelling. Clinically, the lameness due to hock joint and foot pad swelling was particularly seen in BP male birds in nearly all flocks investigated (PCR positive and seropositive for MS). Female birds, although serologically positive for MS, did not show signs of lameness. Moreover, swelling of the hock joint was mild in female birds. The MS-affected BP flocks reported varied morbidity and mortality. The morbidity reached up to 100 per cent in male BP flocks with mortality up to 10 per cent (Tables 1 and 2).

Figure 1: BP birds showing lameness due to MS infection.

The female birds from two CL flocks positive for MS (by PCR and serology) showed lameness as a prominent clinical sign. The swelling of the hock joint in these affected female birds was mild with comparatively a lesser amount of joint fluid. Few affected female birds showed injury to the joint with the open wound possibly due to rubbing of joint to cages or external hard objects. Joint fluid was cloudy to turbid and slimy in most of the affected birds. Paleness of the comb particularly in CL flocks was a constant feature in MS-affected flocks. The other signs included emaciation, dehydration, debility, tracheal rales and ruffled feathers. The morbidity in the CL flock (MS positive by PCR) was 0.42 with negligible mortality (Table 2).

Gross pathology

Grossly, MS-affected BP birds showed moderate to severe unilateral or bilateral hock joint swelling (Figure 2) with occasional involvement of the foot pad (FP) joint (Figure 3). The swollen joint contained turbid to caseous exudate in most birds and blood-tinged fluid in a few birds. The tendons were moderately swollen in acute cases and showed adhesion to the skin in chronic cases. The joint contained turbid to caseous exudate (Figure 4). Breast blister was the most common feature in MS-affected birds. The birds of CL flocks showed mild swelling of the hock joint with a comparatively lesser amount of turbid to caseous exudate. In addition, the birds of the CL flock affected with MS showed moderately cloudy and thickened abdominal air sacs (Figure 5) and accumulation of caseous exudate in the abdominal cavity (Figure 6).

Figure 2: Bilateral swelling of hock joint in MS affected BP birds.

Figure 3: BP birds showing unilateral footpad joint swelling due to MS infection.

Figure 4: MS affected BP bird showing caseous exudates in joint cavity.

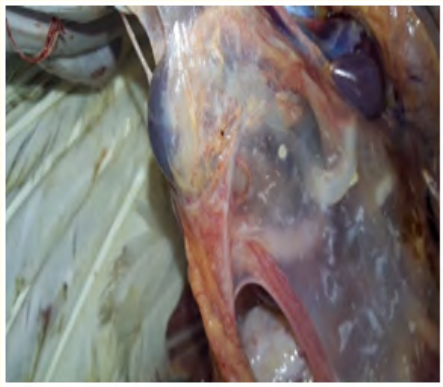

Figure 5: Thicken and cloudy abdominal air sac in MS affected CL birds.

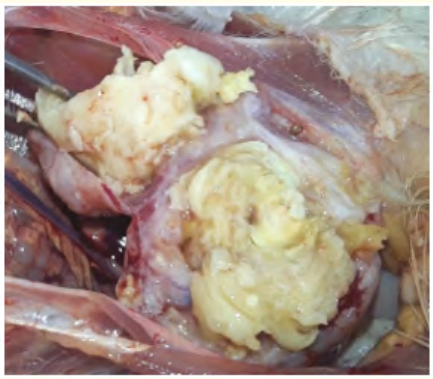

Figure 6: MS affected CL bird showing caseous exudates in abdominal cavity.

Histopathology

Histopathological changes in tendons from MS-affected birds showed oedema, coagulative necrosis, hyperplasia of synovial membranes and sheath, and infiltration of heterophils and lymphocytes (Figures 7, 8, 9 and 10). In chronic cases, villus formation of the synovial membrane with extensive fibrosis in the tendon sheath was observed in most examined birds (Figure 11). Perivascular lymphoid infiltration was the most common feature around the blood vessels in the tendon sheath of MS-affected flocks (Figure 12). Multinucleated giant cells were noticed in birds of a few flocks investigated (Figure 13). Other changes in tendons included a varied degree of fibrous tissue and reticular cell proliferation.

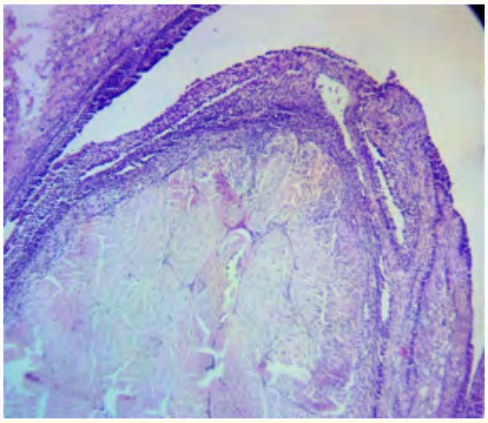

Figure 7: Photomicrograph of tendon showing thickening and hyperplasia of synovial membrane and tendon sheath with cellular infiltration (H & E x 100).

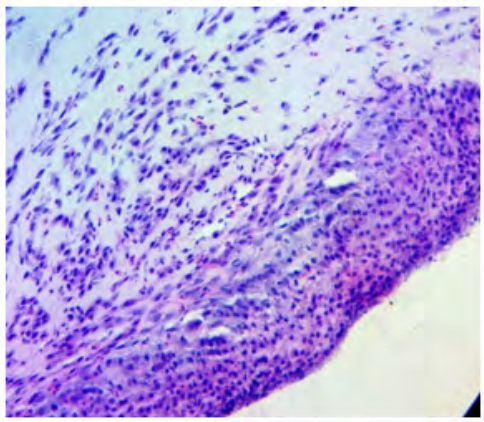

Figure 8: Photomicrograph of tendon showing oedema and infiltration by heterophils and lymphocytes (H & E x 400).

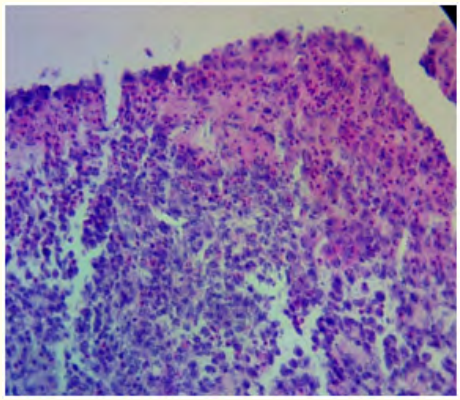

Figure 9: Photomicrograph of tendon showing predominant population of heterophils and mild lymphocytes infiltration (H & E x 400).

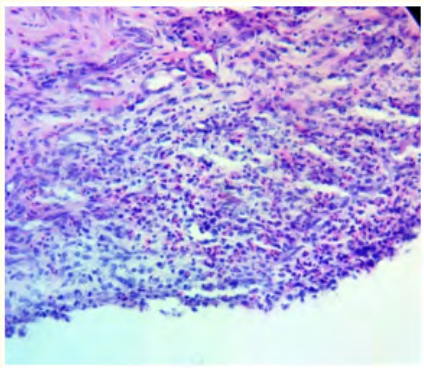

Figure 10: Photomicrograph of tendon showing infiltration by heterophils lymphocytes and mild fibrous tissue proliferation (H & E x 400).

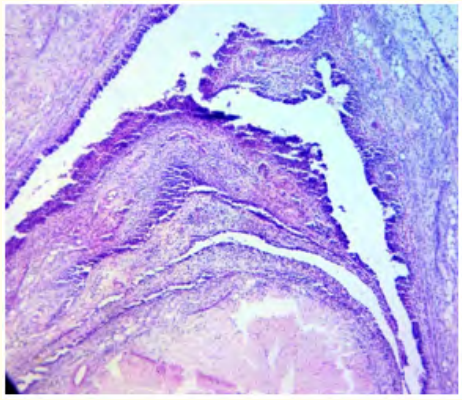

Figure 11: Photomicrograph of hock joint showing sheath hyperplasia with villus formation (H & E x 100).

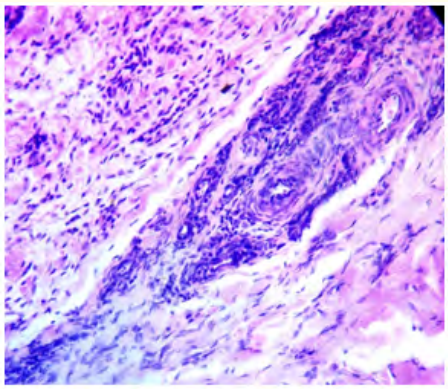

Figure 12: Photomicrograph of hock joint showing perivascular lymphoid cell infiltration (H& E x 400).

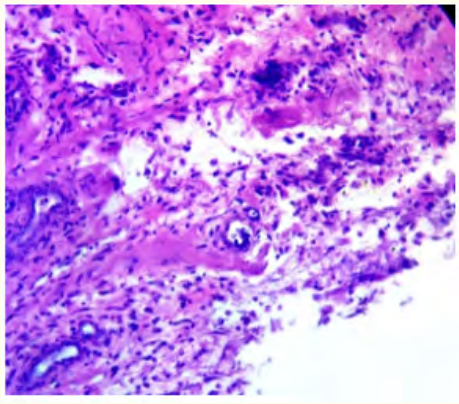

Figure 13: Photomicrograph of hock joint showing multinucleated giant cell in tendon sheath (H & E x 400).

Section of trachea showed mild tracheitis with submucosal edema and mononuclear cell infiltration in the mucosa, particularly lymphocytes and mild sloughing of epithelial cells. Lung showed varied changes from alveolar oedema to interstitial infiltration of heterophils and lymphocytes. Section of the heart showed the predominant population of heterophils and a sparse population of lymphocytes (Figure 14). Occasionally lymphocytic aggregation in cardiac muscle was also noticed. Sections of the liver showed perivascular and periportal infiltration of heterophils and lymphocytes (Figure 15). Occasionally individualization of hepatocytes with the multinucleated giant cell was observed (Figure 16). Brain showed perivascular infiltration of mononuclear cells, particularly lymphocytes and thickening of arteries due to fibrous and reticular cell proliferation.

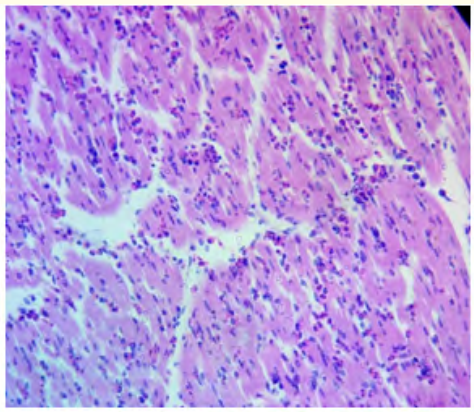

Figure 14: Photomicrograph of heart showing infiltration by heterophils and lymphocytes (H & E x 400).

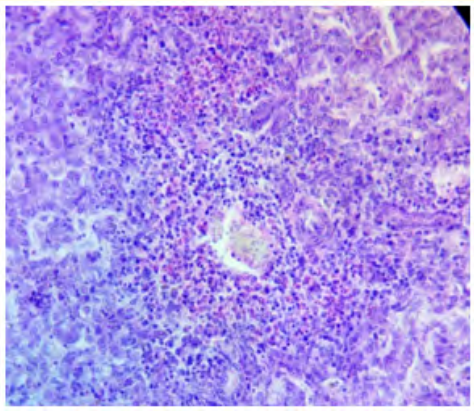

Figure 15: Photomicrograph of liver showing perivascular lymphocytes and heterophils infiltration (H & E x 400).

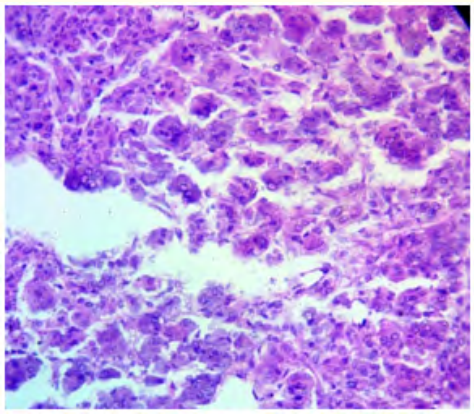

Figure 16: Photomicrograph of liver showing individualization of hepatocytes with giant cell formation (H & E x 400).

Serology

The serum samples from BP and CL flocks affected with tenosynovitis were collected and tested for MS antibodies by ELISA for serological diagnosis. Total of 282 serum samples were tested from 15 BP Farms and two CL farms out of which 183 (64.89%) serum samples were positive for MS antibodies (Table 3). The adult birds (>20 wks age) were more commonly affected than the birds of young age (<20 wks age) group with 89.17 and 34.4 percent sero-positive for MS, respectively.

Farm no.	Farm details	Tested	Positive
1	AR-1A, BP, M, 14 wks	15	00
2	AR-1B, BP, F, 14 wks	17	00
3	AR-06, BP, M, 28 wks	11	11
	AR-06, BP,F, 28 wks	04	04
4	AR-7, BP, M, 31wks	08	00
	AR-7, BP, F, 31 wks	03	00
5	AR-8A, BP, M, 9.3 wks	13	13
6	AR-8B, BP, M, 15.3 wks	15	15
7	AR-8C, BP, M, 21.2 wks	11	11
	AR-8C-1, BP, F, 21.2 wks	14	14
	AR-8C-2, BP, F, 21.2 wks	14	14
8	AR-8D, BP,M, 24.3 wks	17	17
	AR-8D, BP, F, 24.3wks	03	03
9	AR-8E, BP, M, 47 wks	20	20
10	AR-9A, BP, M, 17 wks	14	00
	AR-9A, BP, F, 17 wks	15	04
11	AR-9B, BP, M, 21.2 wks	10	08
	AR-9B, BP, F, 21.4 wks	10	06
12	AR-11, BP, F, 58 wks	10	10
13	AR-12, BP, F, 12wks	10	10
14	AR-13, BP, F, 2 wks	10	01
15	AR-14, BP, F, 3.1 wks	16	00
16	AR-10, CL, 58 wks	10	10
17	AR-2, CL, 55 wks	12	12
	Total	282	183
	Young birds (<20 wks)	125	43 (34.4%)
	Adult birds :BP& CL-(Above 20 wks)	157	140 (89.17)

Table 3: Details of serological diagnosis of MS in breeder and commercial poultry farms.

BP-Broiler parent; CL-Commercial layer; T- Total number of serum sample tested; P- Number of sera positive for MS antibody.

Note: The cutoff point of 1076 and above ELISA titre were considered positive for MS.

Ultrastructural studies

Out of seven tendon specimens examined by TEM, six tendon specimens showed mycoplasma like organism (MLO) in the cytoplasm of fibroblast, macrophages and adipose cells and joint fluid collected from birds affected with tenosynovitis which were confirmed as *Mycoplasma synoviae* by PCR. The morphology of organisms was pleomorphic and varied from round to pear shape with a granular appearance in a few organisms. The organism was more numerous in joint fluids as compared to TEM sections of tendons collected from tenosynovitis-affected birds (Figure 17). However, fibroblasts, macrophages or adipose cells did not show significant alterations in structure. The other ultrastructural changes included unorganized fibrils, severe congestion and infiltration of macrophages, lymphocytes and granulocytes.

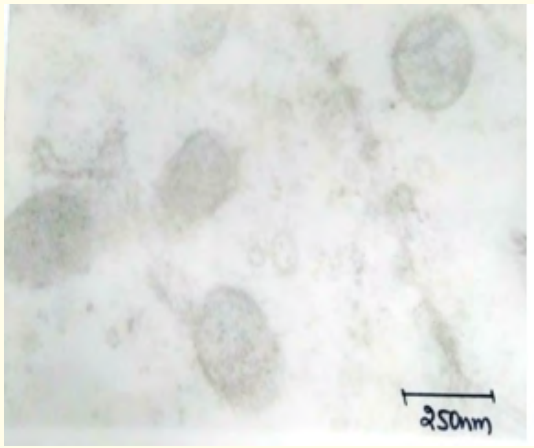

Figure 17: Transmission electron micrograph- Joint fluid showing round and pleomorphic mycoplasma like organism (MLO) (UA & LC x 77200).

Detection of *Mycoplasma synoviae* by PCR in tenosynovitis-affected flocks

In order to investigate the cause of tenosynovitis in parent flocks, tissues from 19 parent flocks and two CL flocks showing signs of lameness due to tenosynovitis were tested by conventional PCR, out of which samples from three flocks (one CL and two BP) were amplified and detected by PCR using VlhA gene-specific primers. The band size of the amplicons of 350-400 bp characteristic of MS was detected and matched with a standard DNA ladder of 100bp as well as a positive control of MS when run in 2 % agarose gel containing ethidium bromide (Figure 18).

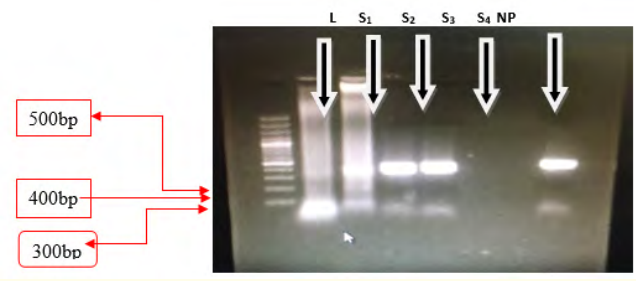

Figure 18: Detection of MS by PCR: Lane L-100 bp to 1 kb; S1 to S4: Samples 1 to 4; N: Negative control; P: positive control.

Discussion

The MS affected broiler parents birds showed lameness due to hock joint and footpad swelling in male birds. Interestingly, the female birds in contact with infected male birds (housed in the single shed) did not show any signs of lameness, although the female birds were serologically positive for MS. However, lameness was observed in the female birds from the CL flock with no to mild hock joint swelling. The paleness of the comb particularly in CL flocks was a constant feature. The morbidity reached up to 100 per cent in male BP flocks with 10 per cent mortality. The morbidity in the MS-affected CL flocks was up to 0.42 per cent with non-significant mortality.

The clinical signs observed in the present study were in close agreement with the clinical signs reported in naturally MS-affected flocks by various authors [7-11]. However, they have reported morbidity ranging from 2 to 75 per cent, usually reaching 5 to 15 per cent, and mortality of 1 and 10 per cent [10,11] and have been attributed to variation in virulence of MS strain. However, very high cumulative mortality (24.49 and 25.55 per cent) was reported in a natural outbreak of mycoplasmal arthritis in broilers [9].

Clinical signs such as emaciation, dehydration and debility observed in the present study could be due to the inability of lame birds to reach feeders and waterer which was also suggested earlier [9].

The clinical symptoms of *Mycoplasma gallisepticum* (MG) infection are generally seen as more severe in males than in females [12]. However, there has been no report on the manifestation of exacerbated clinical signs in male birds due to MS as observed in the present study, and this could possibly be due to breed predisposition or male sex hormone. Pale comb indicating anaemia was observed in MS-affected flocks has also been reported by various authors [7,12-14]. Interestingly, anaemia was observed in the commercial layer flock but not in the BP flocks. The cause of anaemia reported in MS infection has not been well elucidated and researchers suspected haemolysis as a cause of anaemia. However, the deposition of haemosiderin in the liver and spleen was not a constant feature [7]. Moreover, an autoimmune mechanism has been ruled out [7], but the existence of rheumatic factor has been demonstrated in a field outbreak of infectious synovitis [15].

Many authors were of the opinion that the respiratory signs observed in MS infections were similar to the signs shown in MG outbreaks but occur with less severity as a result of infection by MS strain of respiratory tropism [7,11].

In BP flocks, swelling of the hock and FP joint with cloudy to turbid, slimy semi-viscid exudate in most birds and blood-tinged exudate in a few affected birds were noticed. In MS-affected CL flocks, in addition to a swelling of the hock and FP joint, a cloudy and thickened air sac and accumulation of caseous masses in the abdominal cavity were noticed with the dehydrated and emaciated carcass. Breast blister was a prominent feature in MS-affected flocks.

These lesions observed in the present study are in accordance with observations reported by earlier research groups in natural MS infection [7,9-11] and in experimentally reproduced disease [8,16-18].

The lesions in the respiratory tract and air sacs develop due to MS strain which has tropism to the respiratory system and joints [10,11].

Microscopic changes in tendons from MS-affected birds were oedema, coagulative necrosis, and infiltration of heterophils and lymphocytes in acute cases and synovial membrane and sheath hyperplasia with villus formation and infiltration of heterophils, lymphocytes and macrophages in chronic cases. Perivascular lymphoid infiltration, multinucleated giant cells and lymphoid aggregations in various organs including the liver and brain were noticed. Organs like the trachea, air sac and lung showed mild to moderate inflammation. The histopathological lesions observed were in accordance with observations reported in natural outbreaks [7,10,11], and in an experimental study [8,16,17,19] of MS infection. Similar to the present study, perivascular lymphocytic cell infiltration seen around blood vessels of the tendon sheath and surrounding skeletal muscle as well as around blood vessels of the portal triad in the liver was found to be characteristic lesion and newer lesion reported in natural infection of MS and was reported in experimental infection of MS [19].

Out of seven tendon specimens examined by EM, six tendon specimens showed MLO (MS) in the cytoplasm of fibroblast, macrophages and adipose cells. The morphology of organisms was pleomorphic and varied from round to pear shape. The ultra-structural changes included unorganized fibrils, severe congestion and infiltration of macrophages, lymphocytes and granulocytes. The present observations were in accordance with earlier studies, who have also reported similar ultrastructural lesions in the tendons of birds inoculated with MS [20].

In order to investigate the cause of tenosynovitis in parent flocks, tissues from 19 parent flocks and two CL flocks showing signs of lameness due to tenosynovitis were tested, out of which three samples were amplified and detected by conventional PCR using the VlhA gene-specific-primers. In the present study, MS was detected in less number of flocks by PCR as compared to serological study (64.89%) on MS. The earlier study reported that certain inhibitory substances in samples results in less number of PCR-positive results from seropositive MG-affected flocks. Another reason could possibly be the time gap between the collection of samples after the outbreak, the collection of samples on ice without MS PPLO broth as well as the long interval between the collection of samples and their testing by PCR.

Similarly, diagnosis of MS by PCR from chickens of commercial and Government poultry farms from different parts of Karnataka, Andhra Pradesh and Tamil Nadu using samples inoculated in Frey's mycoplasma broth was carried out in which three samples out of 153 samples yielded an amplicon size of 207 bp specific for MS [22]. In another study using duplex PCR assay targeting the Intergenic Spacer Region of MG and 16S rRNA gene of MS for molecular detection of avian mycoplasma directly from tissues of poultry affected with respiratory infections in Haryana was conducted [23]. Out of 92 different poultry flocks affected with respiratory infections and tested by duplex PCR assay, 25 flocks (27%) were positive for MG, two (2.1%) for MS and one (1.08%) for both MG and MS [23]. Marginally higher incidence of MS was reported by Tawfik and co-author [24] in which they attempted 60 samples for isolation MS from broiler chicken with arthritis symptoms and cultivated on PPLO media for isolation of MS. Out of 60 samples, 6 (10%) were positive for MS. Amplification of the vlhA gene of MS by PCR showed that out of 6 isolates, 3 (50%) were positive for the gene.

In another study, authors collected 365 tracheal swabs from each seropositive flock for PCR to determine the presence of MS

organisms. The PCR test was applied using specific primers to amplify the 214 bp region of the 16S rRNA gene of the organism. In PCR, all seropositive flocks showed a positive result for MS [25].

Conclusions

Mycoplasma synoviae was identified as a major cause of lameness due to tenosynovitis in broiler parent flocks in Telangana and Maharashtra. Serological testing showed 183 out of 282 (64.89%) serum samples were positive for MS antibodies, with higher seropositivity in adult birds. PCR detected MS in 3 out of 21 samples, while transmission electron microscopy revealed Mycoplasma-like organisms in 6 of 7 tendon samples. Clinical signs were mainly observed in male breeder birds. Common gross lesions included pale comb, breast blister, egg peritonitis, and tenosynovitis. Histopathological findings included perivascular cuffing of lymphocytes and heterophils, giant cell formation in tendons, and hepatocyte individualization, along with previously reported characteristic lesions in tendons and visceral organs.

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